

Amendments to the Claims

Please amend the claims as indicated in the following listing of claims. This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1. (Currently amended) A method of mixing heterologous genes in expression cassettes located on artificial chromosomes said method comprising the steps of:

(a) providing two initial populations of cells that can mate or fuse with each other, said initial populations comprising at least [[2]]two cells in each population, and at least two cells in one population having different combinations of heterologous genes and/or different combinations of expression cassettes,

each cell comprising at least a first type of artificial chromosome, the at least first type of artificial chromosome comprising both (i) a concatamer of at least two expression cassettes comprising heterologous genes and (ii) at least one selectable marker, the selectable markers being allocated to artificial chromosomes so that each type of artificial chromosome from each population can be individually selected for,

the concatamer comprising, in the 5' → 3' direction, a nucleotide sequence of the general formula:

$[rs_2\text{-}SP\text{-}PR\text{-}X\text{-}TR\text{-}SP\text{-}rs_1]_n$

wherein

rs₁ and rs₂ together comprise a restriction site, denoted rs₁-rs₂, comprising a recognition sequence of 7 to 50 bases where substantially all restriction sites are complete restriction sites recognized by the same enzyme,

SP denotes a spacer of at least two nucleotide bases,

PR denotes a promoter, capable of functioning in a cell,

X denotes an expressible nucleotide sequence,

TR denotes a terminator, and

SP individually denotes a spacer of at least two nucleotide bases, and

$n \geq 2$, and

wherein at least a first cassette is different from a second cassette; and

(b) mating or fusing the cells with each other, and selecting mated or fused cells that
carry at least a subset of the selectable markers present on the artificial chromosomes in
the two initial populations.

2. (Original) The method according to claim 1, further comprising causing the selected mated cells to undergo meiosis.

3. (Cancelled)

4. (Currently Amended) The method according to claim 1, wherein the subset of markers selected for comprises at least one marker from ~~and~~ an artificial chromosome in each of the initial populations to ensure selection of mated or fused cells.

5. (Currently Amended) The method according claim 1, wherein the selection for a subset of the selectable markers includes selecting at least 70% of all diploid types present in the mated or fused population.

6. (Cancelled)

7. (Currently Amended) The method according to claim 1, further comprising screening mated cells for one or more parameters related to a desired functionality(ies) and selecting cells having a predefined selection criterion(a) to undergo meiosis and mating or further fusion.

8. (Currently amended) The method according to claim ~~[[1]]~~2, further comprising screening cells that have undergone meiosis for at least one parameter related to a desired functionality(ies) and selecting cells having a predefined selection criterion(a) to undergo mating and meiosis.

9. (Currently Amended) The method according to claim 8, wherein the selection threshold(s) associated with the desired functionality(ies) is increased for each round of mating and meiosis or fusion.

10. (Canceled)

11. (Currently Amended) The method according to claim 2, further comprising ~~repeating the steps of claim 2 at least twice~~ mating the cells that had undergone meiosis, selecting mated cells that had undergone meiosis, and causing the selected mated cells that had undergone meiosis to undergo further meiosis.

12.-14. (Canceled)

15. (Currently Amended) The method according to claim ~~[[1]]~~2, further comprising separating cells of the two mating types from each other after meiosis.

16. (Currently Amended) The method according to claim 1, further comprising mixing spores from different populations into said cell population prior to mating.

17. (Cancelled)

18. (Previously presented) The method according to claim 2, further comprising adding a further population of cells with types of artificial chromosomes comprising at least two expression cassettes with heterologous genes, the cells being capable of mating with the cells that have undergone mating and meiosis, the further population comprising at least 2 cells with combinations of expression cassettes different from the combinations in the cells of the initial

population, the artificial chromosomes of said further population carrying at least one selectable marker.

19. - 22. (Canceled)

23. (Currently Amended) The method according to claim 1, wherein at least one of the two initial populations of cells that can mate or fuse with each other further carry at least a second type of artificial chromosome with expression cassettes comprising heterologous genes, the first and second types of artificial chromosome carrying at least one selectable marker so that said first and second type of artificial chromosome can be individually selected for.

24. - 25. (Canceled)

26. (Currently Amended) The method according to claim 1, wherein the two initial populations of cells that can mate or fuse with each other carry from 1 to 10 types of artificial chromosomes, each type of artificial chromosome of each population carrying at least one selectable marker so that each of the types of artificial chromosomes from each of the two populations can be individually selected for.

27. (Cancelled)

28. (Currently amended) The method according to claim 1, wherein each cell carries [[2]]two artificial chromosomes per cell that can mate~~[[,]]~~or fuse.

29. (Cancelled)

30. (Previously Presented) The method according to claim 1, wherein each artificial chromosome carries at least two selectable markers, the selectable markers being allocated to

artificial chromosomes so that each type of artificial chromosome from each population can be individually selected for.

31.-35. (Cancelled)

36. (Previously Presented) The method according to claim 1, wherein the two initial populations are of different mating types.

37-38. (Cancelled)

39. (Previously Presented) The method according to claim 1, wherein type of artificial chromosomes with the same marker or combination of markers differ with respect to combinations of expression cassettes comprising heterologous genes.

40. - 42. (Cancelled)

43. (Currently Amended) The method according to claim 1, wherein the species of cells is spore-forming fungal cells ~~selected from a spore-forming species.~~

44. (Previously Presented) The method according to claim 1 wherein the species of cells is yeast cells.

45. - 48. (Cancelled)

49. (Currently Amended) The method according to claim 1, wherein the mated or fused cells are diploid or tetraploid or hexaploid.

50.-53. (Cancelled)

54. (Previously presented) The method according to claim 1, comprising nucleotide sequences from at least two expression states.

55-56. (Cancelled)

57. (Previously Presented) The method according to claim 1, wherein substantially all expression cassettes on one artificial chromosome are different.

58. (Currently Amended) The method according to claim [[52]] 1, wherein at least one expression cassette comprises an intron between the promoter and the expressible nucleotide sequence.

59. (Previously Presented) The method according to claim 1, wherein the different combinations of expression cassettes comprises different promoters, and/or different expressible nucleotide sequences, and/or different spacers and/or different terminators and/or different introns.

60. (Currently Amended) The method according to claim [[52]] 1, wherein n is at least 10[[,]].

61. (Cancelled)

62. (Currently Amended) The method according to claim 54 1, wherein the ~~different expression states represent~~ nucleotide sequences originate from at least two different tissues; ~~such as at least two organs, such as at least two species, such as at least two genera.~~

63.-79. (Cancelled)

80. (Currently amended) The method according to claim 1, further comprising subjecting the populations of cells to physical isolation of artificial chromosomes from the populations for every 2-3 4-5 rounds of meiosis and selection or fusion, and transferring the isolated artificial chromosomes into new host cells.

81-101. (Cancelled)

102. (Previously Presented) The method according to claim 1, wherein at least two cells in each population has different combinations of heterologous genes and/or different combinations of expression cassettes.

103. (New) The method according to claim 1 wherein each artificial chromosome further comprises a conditional centromere.

104. (New) The method according to claim 2, further comprising selecting, among cells that have undergone meiosis, cells that carry at least a subset of the selectable markers present on the artificial chromosomes in the two initial populations.